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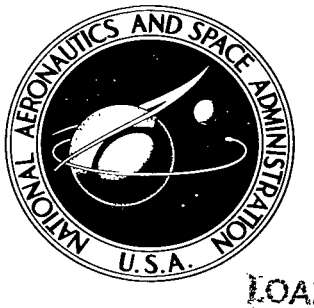

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by N. N. Zhukov-Verezhnikov, et al.

Paper presented at the XV International Astronautical Congress
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RESULTS OF MICROBIOLOGICAL AND CYTOLOGICAL INVESTIGATIONS ON VOSTOK TYPE SPACECRAFT

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ABSTRACT

Numerous ground-based experiments showed that lysogenic bacteria by virtue of their pathological bacteriophage information are highly sensitive to ionizing radiation and ultraviolet rays. It is a well-known fact that human cells in monolayer cultures are also sensitive to these factors. Therefore, beginning with the second spacecraft and thereafter on vehicles of the Vostok type, experimental use was made of the lysogenic bacteria E. Coli K-12 (λ), cultures of normal and cancerous human cells (strains of fibroplasts, amnion, Hela), and some other biological objects.

The experiments showed that increase in length of flight is associated with intensified bacteriophage production by the lysogenic culture and slight changes in the nature of the growth of the normal and cancerous cell cultures.

The biological effect noted in the experiments on Vostoks 3, 4, 5, and 6 seems to have been caused by a set of space flight factors, radiation and vibration in particular. As was demonstrated in the ground-based experiments, vibration helped to sensitize cells of the lysogenic culture to gamma irradiation (Co^{60}).

Another source of the genetic changes may have been weightlessness combined with other space flight factors. Elucidation of the genetic effect of these factors will be exceptionally valuable in carrying out the biomedical program for longer space flights.

The recent progress made in microbiology and the study of clonal lines of cultures of human and animal cells are contributing to the solution of many problems in medical genetics. Specifically, microbiological and cytological objects are being increasingly used to simulate hereditary anomalies caused by the interaction of environmental

mutagenic factors (Refs. 4, 6). Hence the use of such models to study the "genetic situation" in space has been extremely valuable, especially in connection with the program for conquering outer space.

The biological objects that have participated in space experiments (lysogenic bacteria, cultures of human cells--Hela strains, amnion, fibroblasts) are useful both as biological monitors of space radiation and as unique genetic indicators recording the factors that are capable (at the cellular level) of causing hereditary changes of pathological character. One of these genetic indicators was the lysogenic culture E. Coli K-12 (λ), which owing to its high sensitivity to ionizing radiation was a striking example of an hereditary anomaly apparently of the transformation type (Ref. 4). This is evidenced by a mass of experimental data concerning the biological effect of various kinds of ionizing radiation on the lysogenic strain of the intestinal rod K-12 (λ) (Refs. 5, 7, 8, 10). It was shown that the lysogenic bacteria had only to be exposed to small doses of gamma rays (of the order of 0.3-0.5 r) protons with an energy of from 126-660 Mev, or rapid neutrons with an energy of from 0.1-100 Kev (with a minimum irradiation dose of 0.3 rad) for the genetic system of prophage to be irreversibly activated in the direction of forming infectious bacteriophage with a lethal outcome for the bacterial cell. Consequently, as noted above bacteriophage information is a potentially lethal pathological character resulting in the bacterial cell becoming exceptionally sensitive to mutagenic factors as compared with all the biological objects presently known.

The studies of French investigators (Refs. 11, 12, 13) and numerous experiments carried out in the Institute of Experimental Biology, USSR Academy of Medical Sciences, revealed that the degree of induction of bacteriophage production by a lysogenic culture is directly related to the dose of gamma radiation. This is of considerable significance in quantitative expression of the biological effect of penetrating radiation. Similar results were obtained by irradiating a lysogenic culture with high-energy protons (Refs. 5, 8). Finally, it was found in special investigations that lysogenic bacteria, even when frozen (-30° to -45°C), are capable of dose cumulation during exposure to gamma radiation (Co^{60}) at a dose rate of 4 r/24 hours. The biological effect of gamma rays is noticeable with as small a dose as 0.2 r, although a statistically significant increase in induced bacteriophage production resulting from the cumulative effect of ionizing radiation begins with 0.8 r.

Thus, ground-based radiobiological investigations on lysogenic bacteria served as the basis for using this model as a biological monitor of the genetic effect of space flight factors.

It is known that human cells in monolayer cultures are also sensitive to ionizing radiation. Therefore, starting with the second spacecraft and in the experiments thereafter, normal and cancerous human cell

cultures (strains of fibroblasts, amnion, Hela cells) as well as lysogenic bacteria were used.

The experiments showed that on the historic flight of Yu. A. Gagarin and G. S. Titov there were no factors that impaired the viability and original growth potential of human cells in tissue culture.

The data obtained in the experiments on the satellites were completely confirmed after the first flight of Vostok 1 (Refs. 2, 3). This means that cultures of human cells exposed to space flight factors did not differ significantly from cultures left on earth with respect to such indicators as proliferation rate, percentage of lethality, and morphological, antigenic, and cultural properties.

However, analysis of specimens of lysogenic bacteria after the flight of Vostok 2 revealed that the number of bacteriophage particles in the experiment was 1.2 times larger than in the control. Even though the discrepancy was slight (and statistically insignificant) as compared with the amount of spontaneous bacteriophage production by the control, there was nevertheless some tendency for the bacteriophage-producing activity of the lysogenic culture E. Coli K-12 (λ), carried in spacecraft to increase.

This was subsequently confirmed by experiments on the spacecraft that completed longer flights. For example, investigation of specimens exposed to space flight factors on Vostok 3 showed that the number of bacteriophage-producing cells found after the craft landed exceeded the spontaneous level of bacteriophage production 4.6 fold (degree of significance with this difference: 0.2 percent). Analysis of specimens of lysogenic bacteria exposed to space flight factors on Vostok 4 showed that the number of bacteriophage particles exceeded the spontaneous level of the control group (left at the space field) 1.9 fold.

Thus, the inducing effect on the intestinal rod K-12 (λ) was more pronounced (2.4 fold) in the experiments on Vostok 3 than in those on Vostok 4. This is apparently related to the more prolonged effects of space flight factors on Vostok 3.

The significance of more prolonged exposure is also underlined by the data obtained in investigations on the biological effect of space flight factors on normal and cancerous cells in tissue culture. Specifically, it was found that after repeated exposure of cultures of human Hela line cells to space flight factors on Vostok 4 and Vostok 6 there was a longer latent period for restoration of growth capacity than in the cells that had been carried in space once or were left under laboratory conditions as a control. The difference showed up very clearly when evaluating the proliferation rate (number of times the cells grown in culture increased to the number of those sown), which was almost

half that of the culture of human Hela cells carried in space on two occasions (Vostok 4 and Vostok 6) as compared with an intact line of cells or with a laboratory culture that had been in space once (on Vostok 4). These data tend to support the possibility that the biological effects of space flight factors on normal and cancerous human cells in tissue culture are cumulative, although the causes and nature of the observed changes are not at all clear and require further investigation.

However, a comparison of the results of experiments on lysogenic bacteria exposed to space flight factors on Vostok 3 and Vostok 4 with those obtained from experiments on Vostok 5 and Vostok 6 failed to reveal any direct connection between the biological effects and the time of exposure in space. For example, despite the greater length of the Vostok 5 flight, the degree of induction of the lysogenic bacteria was actually the same as in the experiments on Vostok 3. Thus, it is fair to deduce from our experiments that on the flights of these spacecraft, factors were actually present which caused a genetic effect in that they increased the bacteriophage-producing activity of the lysogenic culture. We must emphasize, moreover, that the slight variations in degree of induction obtained in the experiments on lysogenic bacteria obviously show that the inducing factors connected with the flights of Vostoks 3, 4, 5, and 6 are similar.

Despite a number of procedural matters connected with the running of experiments on spacecraft, the results are useful in analyzing the experimental material from the standpoint of elucidating the reasons for the inducing effect noted in lysogenic bacteria during space flights. The mean value of induction of a lysogenic culture on the flights of Vostok 3 and Vostok 5 was approximately 3 units, which is equivalent to the inducing effect of 3.2 r of gamma rays. On Vostok 4 and Vostok 6 the degree of induction was 1.8 units, which is equivalent to the inducing effect of about 0.8 r of gamma rays. It is known from the readings of the physical instruments that these doses are in excess of the radiation level during space flights. Therefore, the observed genetic effect may have been caused by some other factors that arose during the flights--weightlessness, or such mechanical agents as accelerations and vibrations. The latter were of most interest. Accelerations, as previously demonstrated, did not cause any inducing effect in the general complex of space flight factors. Accordingly, specimens of the lysogenic culture E. Coli K-12 (λ) were exposed in the laboratory to vibrations in a frequency range of 18, 35, 75, 100, and 700 cps for 15, 30, and 60 minutes (accelerations of 10 g). In parallel experiments vibrations were combined in various ways with gamma irradiation from a Co^{60} source in doses of up to 100 r at the rate of 21 r/min.

In accordance with the established method (Refs. 7, 9), both the number of viable cells and the number of bacteria-producing bacteriophage were determined in the control and experimental specimens simultaneously.

The results were evaluated from the degree of induction (R), which reflects the ratio of the level of induced bacteriophage production by the experimental lysogenic culture to the amount of spontaneous bacteriophage production by the control specimens.

The number of bacteriophage particles in the experimental specimens did not exceed the level of spontaneous bacteriophage production by the control. On the contrary, in several experiments "R" was below unity, an indication that spontaneous bacteriophage production by E. Coli K-12 (λ) had been somewhat inhibited. It will be noted that the amplitude of variations in these ranges and the duration of exposure to vibrations had no significant effect on "R", which fluctuated between 0.8 and 1.1.

The results were different when the lysogenic culture was exposed to vibrations combined with gamma irradiation. For experiments in which lysogenic bacteria were exposed to vibrations for 15 minutes, irradiated, and then again exposed to 15 minutes of vibrations, the number of induced microbial cells was 1.4 times greater than the number of bacteria producing bacteriophage after gamma irradiation alone. This difference proved on statistical processing to be significant within 0.1 percent.

A slight increase in the inducing effect of gamma rays was also noted after a single exposure to vibrations 1-1/2 hours before irradiation. However, vibrations 1-1/2 hours after irradiation, as in the case of vibrations alone, had no effect. The number of induced bacteriophage particles here was almost the same as in the specimens exposed to irradiation alone.

Thus, the resultant data show that vibrations by themselves do not induce bacteriophage production, although they do increase the sensitivity of lysogenic bacteria to subsequent gamma irradiation. This is also suggested by the fact that vibration effects do not exceed the biological effects of ionizing radiation if they are applied after gamma irradiation.

Our investigations warrant the assumption of a possible connection between the genetic effect observed in the lysogenic bacteria subjected to space flight factors on the Vostoks 3, 4, 5, and 6 and the complex effect of these factors, radiation and vibration in particular. Vibration seems to help sensitize the cells of a lysogenic culture to cosmic radiation. This was probably a major element in the discovery that cosmic radiation in doses exceeding our technical capabilities exerted an inducing effect.

Nor can we rule out the possibility that weightlessness combined with other factors (specifically, ionizing radiation) may have been the source of the genetic changes. However, these problems cannot be resolved without additional investigations to determine their biological

effect. They will be of great value in carrying out the biomedical program planned for extended space flights.

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